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IS 14806 : 2000

भारतीय मानक

एजोस्पिरिलम जीवाणु कल्चर

Indian Standard

AZOSPIRILLUM INOCULANTS

ICS 65.080

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BUREAU OF INDIAN STANDARDS
MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG
NEW DELHI 110002

October 2000

Price Group 3

AMENDMENT NO. 1 JUNE 2008
TO
IS 14806 : 2000 AZOSPIRILLUM INOCULANTS

(*Page* 1, *clause* **4.2**, *line* 5) — Substitute '150 to 212 µ (72 to 100 mesh) IS sieve' *for* '100 µ (sieves)'.

(FAD 7)

Reprography Unit, BIS, New Delhi, India

AMENDMENT NO. 2 MAY 2012
TO
IS 14806 : 2000 AZOSPIRILLUM INOCULANTS

[*Page* 1, *clause* **6.1**(h)] — Substitute 'Net quantity in kg and area meant for;' *for* 'Net mass in kg and area meant for;'.

(FAD 7)

Reprography Unit, BIS, New Delhi, India

Soil Quality and Improvement Sectional Committee, FAD 27

FOREWORD

This Indian Standard was adopted by the Bureau of Indian Standards, after the draft finalized by the Soil Quality and Improvement Sectional Committee had been approved by the Food and Agriculture Division Council.

The bacteria of genus Azospirillum is gram negative, microaerophilic and fixes nitrogen in the roots of many non-leguminous crops like cereals, millets, forage crops and vegetables, etc. Besides fixing nitrogen, it also produces growth promoting substances required for vegetative growth of the plants. These bacteria although present in most soil types, the number of Azospirillum and extent of the benefits varies in the soil. Many brands of Azospirillum inoculants are marketed in the country and they have been found to vary in quality.

It is obligatory on the part of the manufacturers to employ qualified Soil Microbiologist/Microbiologist/ Agricultural Graduates/Graduates in Biology trained in soil microbiology in their staff. Manufacturers shall also maintain a quality control laboratory capable of carrying out the tests according to this specification.

For the purpose of deciding whether a particular requirement of this standard is complied with, the final value, observed or calculated, expressing the results of a test or analysis, shall be rounded off in accordance with IS 2 : 1960 'Rules for rounding off numerical values (*revised*)'. The number of significant places retained in the rounded off value should be the same as that of the specified value in this standard.

Indian Standard

AZOSPIRILLUM INOCULANTS

1 SCOPE

This standard prescribes the requirements, method of sampling and tests for Azospirillum inoculants.

2 REFERENCE

The following Indian Standard contains provisions which, through reference in this text, constitute provisions of this standard. At the time of publication, the edition indicated was valid. All standards are subject to revision, and parties to agreements based on this standard are encouraged to investigate the possibility of applying the most recent editions of the indicated below :

IS No.	*Title*
8268 : 1986	Specification for rhizobium inoculants (*first revision*)

3 TERMINOLOGY

3.1 For the purpose of this standard following definitions shall apply.

3.1.1 *Azospirillum Inoculant* (*ASI*) — ASI is a product having large population of a strain or a combination of several strains of Azospirillum for a particular crop or a group of crops which shall develop efficient roots in the plant and enrich soil with nitrogen and thereby enhance nitrogen fixation, crop growth and productivity.

3.1.2 *Group of Crops* — Crops including cereals, millets, forage crops, vegetables, fruits, plantation crops and sugar crops which are supplied nitrogen efficiently by given strain of Azospirillum for a particular crop for a group of which shall develop efficient roots and the plant enrich soil with nitrogen and their by enhance nitrogen fixation crop growth and productivity.

4 REQUIREMENTS

When tested by the method prescribed in Annex A of the standard, ASI shall contain 107 viable Azospirillum cells/g of the carrier material on dry mass basis.

4.2 The powder carrier material such as peat, lignite, charcoal or similar material such as soil: cow dung or soil: compost may be used. It shall be neutralized with calcium carbonate and then sterilized. When tested it shall pass through 100 µ (sieves).

4.3 ASI when tested by the method prescribed in Annex A, shall have no contamination with other micro-organisms at 10^5 dilution. ASI contamination in semi solid medium should be checked by semi dilution and spread plate method with solid complete medium.

4.4 When tested by the method prescribed in Annex B, the *p*H of ASI shall be 6.5 to 7.5.

4.5 Moisture

Azospirillum BI carrier shall be in the form of fribal (moist) with 30-40 percent moisture.

4.6 When tested by the method prescribed in Annex C, ASI shall show effective root development on all cultivar/crops against which the inoculant is intented to be used.

4.7 Specified mother culture be obtained from any institution maintaining the mother cultures. The manufacturer may control the quality of the broth as given in Annex D, it should get verified at least by two institution as mentioned below.

NOTE — At present National Biofertiliser Development Centre (NBDC), Ghaziabad and its six Regional Centres located at Bangalore, Bhubaneshwar, Imphal, Hissar, Jabalpur and Nagpur; Indian Agricultural Research Institute (IARI), New Delhi; Tamil Nadu Agricultural University (TNAU), Coimbatore; University of Agricultural Science, Bangalore; are sources for supplying the mother culture.

5 PACKING

ASI shall be packed in polyethylene packs, thickness which shall not be less than 75-100 micron.

6 MARKING

6.1 Each polyethylene pack shall be marked legibly and indelibly with the following information:

a) Name of the product, specially as Azospirillum inoculant;
b) Name and address of the manufacturer;
c) Crop(s) for which intended;
d) Type of the carrier used;
e) Batch number;
f) Date of manufacture;
g) Expiry date which shall not be less than 6 months from the date of manufacture;
h) Net mass in kg and area meant for;

j) Storage instructions worded as under :
"STORE IN COOL PLACE AWAY FROM DIRECT SUN LIGHT AND HEAT"

k) Any other information required under the *Standards of Weights and Measures (Packaged Commodities) Rules*, 1977.

6.2 Items (c), (f) and (g) shall be printed on a coloured ink background.

6.3 Direction for use of ASI shall be printed briefly on the packets as given in Annex E of the standard. A separate pamphlet may preferably be given with it.

6.4 BIS Certification Marking

The product may also be marked with the Standard Mark.

6.4.1 The use of the Standard Mark is governed by the provisions of *Bureau of Indian Standards Act*, 1986 and Rules and Regulations made thereunder. The details of conditions under which the licence for the use of Standard Mark may be granted to manufacturer's or producers may be obtained from the Bureau of Indian Standards.

6.5 Storage

ASI shall be stored by the manufacturer in a cool and dry place away from direct heat preferably at a temperature of 20° C and not exceeding 30°C. It shall also be the duty of the manufacturer to instruct the retailers and, in turn, the users about the precautions to be taken during storage.

7 SAMPLING

The representative samples of the Azospirillum inoculant (ASI) shall be drawn as prescribed in Annex F of IS 8268.

ANNEX A

(*Clauses* 4.1 *and* 4 .3)

DETERMINATION OF NUMBER OF AZOSPIRILLUM CELLS

A-1 APPARATUS

A-1.1 Pipettes — Graduated (1 ml and 10 ml, capacity)

A-1.2 Conical flasks — 150 ml and 250 ml.

A-1.3 Screw capped tubes of 10 ml capacity.

A-1.4 Incubator

A-1.5 Petridishes

A-1.6 Hot Air Oven

A-1.7 Autoclave

A-1.8 *p*H Meter

A-1.9 Visual counting and use of MPN table (MPN table be better kept framed as an apparatus).

A-2 REAGENTS

A-2.1 Use a medium of the following composition :
NFB Medium (Nitrogen free bromothymol blue medium)

Malic acid	5.0 g
Potassium hydroxide	4.0 g
Di-potassium hydrogen phosphate	0.5 g
Ferrous sulphate	0.05 g
Manganese sulphate	0.01 g
Magnasium sulphate	0.1 g
Sodium chloride	0.2 g
Calcium chloride	0.1 g
Sodium molybdate	0.002 g
Distilled water	1 000 ml
Bromothymol blue (0.5% alcoholic solution)	2.0 ml
Agar	1.7 g
*p*H adjusted to 6.5 - 7.0	

A-2.2 Sterilizing and processing for plate preparation can be done by same procedures as described in **A-2.3** of IS 8268.

A-3 PREPARATION OF SERIAL DILUTION FOR COUNT BY MPN METHOD

A-3.4 Dispense 30 g of ASI in 270 ml of sterile water and shake for 10 minutes on a reciprocal shaker. Make serial dilutions upto 107 dilution. Pipette out 0.2 ml aliquots of 105 to 107 dilution and deliver it to screw cap tubes or petridishes containing set medium as described in **A-2.1**. Spread the aliquots over the plates. Invert the plates and place them in the incubator at 28 ± 2°C for 3 days. Use 5 replicates of 105, 106 and 107 level.

A-4 COUNTING

A-4.1 Count the tubes or plates which have turned blue in colour after inoculation and ascertain the presence of pellicles in undisturbed medium. To determine usual contamination on the same examine doubtful objects carefully.

A-4.2 Count all plates/tubes which have turned blue and consider them for the purpose of calculation. Count such type of tubes/plates and tally this count with MPN table to get the number of cells per gram of the carrier.

$$\text{Azospirillum count/g of carrier} = \frac{\text{MPN table value} \times \text{Dilution level}}{\text{Dry mass of product}}$$

ANNEX B

(*Clause* 4.4)

DETERMINATION OF *p*H AND MOISTURE PERCENTAGE

B-1 *p*H

B-1.1 Make suspension of 20 g of the ASI into 50 ml of distilled water and shake on a rotary shaker for 2 hours. Filter this suspension and determine the *p*H of the filterate with the help of *p*H meter.

B-2 DETERMINATION OF MOISTURE OF BIOFERTILIZER PACKETS (METHOD)

B-2.1 Heat 10 g of sample for 12-16 hours in an air oven at 100-105° C. Cool in a desiccator and weigh. The loss in weight represents the moisture. Calculate the moisture percentage on air dry weight basis, by multiplying the loss in weight by ten.

ANNEX C

(*Clause* 4.5)

TEST FOR EFFECTIVE ROOT GROWTH

C-1 POT CULTURE TEST

For the purpose of 'Pot Culture Test' plant nutrition solution, preparation of solutions and procedure shall be same as described in **C-1.1, C-1.1.1** and **C-1.2.1** of IS 8268.

C-1.2. After three weeks of growth thin down the number of plants in each pot to four uniform plants. At the end of three months, take one set of pot from control and inoculated series and separate the plants carefully from the soil under slow running water. Obtain the number, length and mass of the plant as a whole including branch, seeds if any and roots. At the end of three months, harvest the shoot system (depending on the crop). Dry at 60°C for 48 hours and determine the dry mass. For the above purpose maintain a minimum of four replications or a maximum of 16 replications.

C-1.2.3 Better vigour of roots in inoculated seeds if established from the data when compared to the control, is the confirmation of effectiveness of ASI. If there is 10 percent increase in the plants over the dry mass of uninoculated control without nitrate, it may be concluded that the culture is of required quality.

C-2 METHOD FOR ESTIMATING MPN COUNT FOR AZOSPIRILLUM

a) Add 100 g ASI sample to 900 ml of sterile distilled water.

b) Shake for 10 minutes on a reciprocal shaker.

c) Make ten fold dilution series.

d) Pipette 1 ml of each dilution (from 10-1 to 10-7) to each one of 5 replicates (5 tubes containing nitrogen free bromothymol blue semi-solid malate media for Azospirillum).

e) Begin by taking aliquot from the highest dilution and proceed down the series with same pipette

f) Collect the tubes from 105, 106 and 107 dilution level.

g) Incubate the inoculated series for 2 days at 30°C.

h) Check the characteristic sub-surface while particles (+ or –) in malate semi-solid medium, a change to dark blue colour of the medium.

C-3 CALCULATION

a) To calculate the most probable number of organisms in the original sample, select as P_1 the number of positive tubes in the least concentrated dilution in which all tubes are positive or in which the greatest number of tubes is +ve, and let P_2 and P_3 represent the numbers of positive tubes in the next two higher dilution.

b) Then find the row of numbers in Table 1 in which P_1 and P_2 correspond to the values observed experimentally. Follow that row of numbers across the table to the column headed by the observed value of *P*.

c) The figure at the point of intersection is the most probable number of organisms in the quantity of original sample represented in the inoculum added in the second dilution. Multiply this figure by the appropriate dilution factor to obtain the MPN value.

Table 1 Most Probable Numbers for use with 10 Fold Dilutions and 5 Tubes per Dilution (Cochran, 1950)

		Most probable number for indicated values of P_2					
P_1	P_2	0	1	2	3	4	5
0	0	0.018	0.036	0.054	0.072	0.090	—
0	1	0.018	0.036	0.055	0.073	0.091	0.11
0	2	0.037	0.055	0.074	0.092	0.11	0.13
0	3	0.056	0.074	0.093	0.11	0.13	0.15
0	4	0.075	0.094	0.11	0.13	0.15	0.17
0	5	0.094	0.11	0.13	0.15	0.17	0.19
1	0	0.020	0.040	0.060	0.080	0.10	0.12
1	1	0.040	0.061	0.081	0.40	0.12	0.14
1	2	0.061	0.082	0.10	0.12	0.16	0.17
1	3	0.089	0.10	0.13	0.16	0.17	0.19
1	4	0.11	0.13	0.15	0.17	0.19	0.22
1	5	0.13	0.15	0.17	0.19	0.22	0.24
2	0	0.046	0.068	0.091	0.12	0.14	0.16
2	1	0.068	0.092	0.12	0.14	0.17	0.19
2	2	0.093	0.12	0.14	0.17	0.19	0.22
2	3	0.12	0.14	0.17	0.20	0.22	0.25
2	4	0.15	0.17	0.20	0.23	0.25	0.28
2	5	0.17	0.20	0.23	0.26	0.29	0.32
3	0	0.078	0.11	0.13	0.16	0.20	0.23
3	1	0.11	0.14	0.17	0.20	0.23	0.27
3	2	0.14	0.17	0.20	0.24	0.27	0.31
3	3	0.17	0.21	0.24	0.28	0.31	0.35
3	4	0.21	0.24	0.28	0.32	0.36	0.40
3	5	0.25	0.29	0.32	0.37	0.41	0.45
4	0	0.13	0.17	0.21	0.25	0.30	0.36
4	1	0.17	0.21	0.26	0.31	0.36	0.42
4	2	0.22	0.26	0.32	0.38	0.44	0.50
4	3	0.27	0.33	0.39	0.45	0.52	0.59
4	4	0.34	0.40	0.47	0.54	0.62	0.69
4	5	0.41	0.48	0.56	0.64	0.72	0.81
5	0	0.23	0.31	0.43	0.58	0.76	0.95
5	1	0.33	0.46	0.64	0.84	1.1	1.3
5	2	0.49	0.70	0.95	1.2	1.5	1.8
5	3	0.79	1.1	1.4	1.8	2.1	2.5
5	4	1.3	1.7	2.2	2.8	3.5	4.3
5	5	2.4	3.5	5.4	9.2	16	-

ANNEX D

(*Clause* 4.7)

GUIDELINES ON MAINTENANCE AND PREPERATION OF CULTURE AND QUALITY CONTROL AT BROTH STAGE

D-1 MAINTENANCE OF PURE CULTURES

D-1.1 Maintain pure culture of Azospirillum on nitrogen free bromothymol blue medium and maintain as a semi-solid medium as described in **A-2.1** of this standard.

D-1.2 Transfer a loopful of pure culture to each of the agar culture tube asceptically in an inoculation room and incubate 28 ± 2°C for three days and keep it undisturbed. Always keep pure culture below 5°C.

D-2 PREPARATION OF INOCULUM CULTURE AND MASS CULTURE

Inoculum culture and mass culture of this standard shall be prepared as described in **A-2.1, A-2.2.**

D-3 QUALITY CONTROL TEST RECOMMENDED AT BROTH STAGE

D-3.1 Qualitative Test

D-3.1.1 Check for free from contaminants by preparing slide and observing under microscope.

D-3.1.2 The *p*H of bacterial broth shall normally be between 7.0 to 8.0.

D-3.1.3 Gram staining test shall be carried out as described in **D-4.1.3.1** and **D-4.1.3.2** of IS 8268.

D-3.1.4 See the colour change in the media after 24 hours from inoculation. The colour will change from green to blue.

D-3.1.5 Watch the pellicle just below the surface of the media. It is checked on the third day after keeping inoculated broth undisturbed.

D-3.2 Quantitative Test

D-3.2.1 *Most Probable Number (MPN)*

ANNEX E

(*Clause* 6.3)

DIRECTION FOR USE OF AZOSPIRILLUM INOCULANTS

E-1 The contents of the packet are sufficient enough to the given area to be broadcasted or given seedlings for root dipping depending on the specified crops as denoted on the packet. Mix the inoculants with seeds gently with the minimum amount of water, taking care to avoid damage to seed coat. Dry the inoculated seed under shade over clean surface gunny bag and sow them immediately.

E-2 Use only for the crops mentioned-paddy, Azospirillum brasiliense for other crops like millets, etc, use crop specific strain. Use before the expiry date and do not expose to direct sun light or heat.

E-3 ASI is not a chemical fertiliser hence do not mix inoculated seeds or ASI with agro-chemicals.

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